

Expected Results

Suspensions containing large numbers of bacterial spores are obtained with the use of AK Agar #2.

References

1. Arret and Kirshbaum. 1959. J. Milk Food Technol. 22:329.
2. Richardson (ed.). 1985. Standard methods for the examination of dairy products, 15th ed. American Public Health Association. Washington, D.C.

Availability

BBL™ AK Agar #2 (Sporulating Agar)

Cat. No. 210912 Dehydrated – 500 g

APT Agar • APT Broth

Intended Use

APT Agar is used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content. It is also used for maintaining stock cultures of *Weissella (Lactobacillus) viridescens* ATCC™ 12706 used in the assay of thiamine.

APT Broth is used for culturing *Weissella viridescens* ATCC 12706 used in the assay of thiamine. It is also used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content.

Summary and Explanation

Evans and Niven¹ investigated cultivating the heterofermentative lactobacilli that cause the faded or greenish discoloration of cured

meat products, while Deibel, Evans and Niven² investigated thiamine requiring bacteria, specifically *Lactobacillus viridescens*. Their formulations led to the development of APT Agar and APT Broth.

Historically, the lactic acid bacteria, a group of acid-producing bacteria, included the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus*; currently, taxonomists include a number of additional genera (e.g., *Weissella*).³ These organisms are widespread in nature and are associated with bacterial spoilage of foods such as dairy, meat and vegetable products.³ One use of APT Agar and APT Broth is for cultivating these heterofermentative lactic acid bacteria from food products.³

APT Agar and APT Broth are also used in the microbiological assay of thiamine. In the assay, APT Agar is the maintenance medium that preserves the viability and sensitivity of *Weissella viridescens* ATCC 12706. APT Broth is used for growing *Weissella viridescens* ATCC 12706 and preparing the inoculum.

Principles of the Procedure

APT Agar and APT Broth contain peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate. The manganese chloride, magnesium sulfate and ferrous sulfate provide ions used in replication by lactobacilli. Polysorbate 80 is a source of fatty acids required by lactobacilli. Agar is the solidifying agent in APT Agar.

Formulae

Difco™ APT Agar

Approximate Formula* Per Liter

Yeast Extract	7.5	g
Pancreatic Digest of Casein	12.5	g
Dextrose	10.0	g
Sodium Citrate	5.0	g
Thiamine Hydrochloride	1.0	mg
Sodium Chloride	5.0	g
Dipotassium Phosphate	5.0	g
Manganese Chloride	0.14	g
Magnesium Sulfate	0.8	g
Ferrous Sulfate	0.04	g
Polysorbate 80	0.2	g
Agar	15.0	g

Difco™ APT Broth

Consists of the same ingredients without the agar.

*Adjusted and/or supplemented as required to meet performance criteria.

User Quality Control

Identity Specifications

Difco™ APT Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 6.12%, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have a slight precipitate.

Prepared Appearance: Medium amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 6.12% Solution at 25°C: pH 6.7 ± 0.2

Difco™ APT Broth

Dehydrated Appearance: Light tan, free-flowing, homogeneous.

Solution: 4.62%, soluble in purified water with slight heating. Solution is opalescent when hot. After cooling, is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.

Prepared Appearance: Light to medium amber, clear to very slightly opalescent without significant precipitate.

Reaction of 4.62% Solution at 25°C: pH 6.7 ± 0.2

Cultural Response

Difco™ APT Agar or APT Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus fermentum</i>	9338	10 ² -10 ³	Good
<i>Weissella viridescens</i>	12706	10 ² -10 ³	Good

Directions for Preparation from Dehydrated Product

Difco™ APT Agar

1. Suspend 61.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ APT Broth

1. Suspend 46.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For maintaining stock cultures of *Weissella viridescens* ATCC 12706 prepare a stab inoculation. Prepare stock cultures in

triplicate at monthly intervals. One of the transfers is saved for the preparation of stock cultures. The others are used to prepare inoculum in APT Broth for assay as needed. Following incubation at 35-37°C for 24-48 hours, store stock cultures at 2-8°C.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Evans and Niven. 1951. *J. Bacteriol.* 62:599.
2. Deibel, Evans and Niven. 1957. *J. Bacteriol.* 74:818.
3. Hall, Ledenbach and Flowers. 2001. In Downes and Ito (ed.), *Compendium of methods for the microbiological examination of foods*, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ APT Agar

COMPE USDA

Cat. No. 265430 Dehydrated – 500 g

Difco™ APT Broth

Cat. No. 265510 Dehydrated – 500 g

Acetamide Agar

Intended Use

Acetamide Agar is used in the differentiation of nonfermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

Summary and Explanation

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms.^{1,2} However, few organisms are reported to deaminate acetamide.^{3,4} A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification.⁵⁻⁸ The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater*.⁹

Principles of the Procedure

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa*, *Comamonas acidovorans*, *Achromobacter xylosoxidans* subsp. *xylosoxidans* (*Alcaligenes xylosoxidans*) and *Alcaligenes faecalis* (*odorans*).⁸ Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

Procedure

Inoculate the Acetamide Agar slant with a loopful of culture emulsified in BBL™ Trypticase™ Soy Broth. Incubate inoculated slant at 35 ± 2°C and observe daily for 4 days and again at 7 days before discarding as negative.

Expected Results

Deamination of the acetamide is indicated by a pronounced purplish-red color of the medium.

Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc. Appropriate references may be consulted for further information.^{10,11}

Limitations of the Procedure

Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.

Only about 37% of apocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.¹¹

References

1. Gilardi. 1974. *Antonie van Leeuwenhoek. J. Microbiol. Serol.* 39:229.
2. Stainier, Palleroni and Doudoroff. 1966. *J. Gen. Microbiol.* 43:159.
3. Pickett and Pedersen. 1970. *Can. J. Microbiol.* 16:351.
4. Pickett and Pedersen. 1970. *Can. J. Microbiol.* 16:401.
5. Hedberg. 1969. *Appl. Microbiol.* 17:481.
6. Smith and Dayton. 1972. *Appl. Microbiol.* 24:143.
7. Buhlmann, Vischer and Bruhin. 1961. *J. Bacteriol.* 82:787.
8. Oberhofer and Rowen. 1974. *Appl. Microbiol.* 28:720.
9. Clesceri, Greenberg and Eaton (ed.) 1998. *Standard methods for the examination of water and wastewater*, 20th ed. American Public Health Association, Washington, D.C.
10. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore, Md.
11. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Acetamide Agar

SMVW

Cat. No. 221828 Prepared Slants – Pkg. of 10*

*Store at 2-8°C.